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Chemical and Biological Studies on 1,2-Dihydro-*s*-triazines. XI: Inhibition of Root Growth and Its Reversal by Citrovorum Factor

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Previous communications have described the synthesis (*1, 2*) of a new family of 1,2-dihydro-*s*-triazines and the microbiological (*3*), antitumor (*4*), and antimalarial (*1, 5*) activity exhibited by the derivatives of this series. Antimalarial activity (*6*) and antiprotozoan activity (*7*) have also been reported from other laboratories. The present report (*8*) concerns the activity of a representative compound—4,6-diamino-1-(3′,4′-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (D-54 · HCl) (*1, 2*)—in a turnip seedling system similar to the "Kressewurzel growth test" (*9*).

Seeds of *Brassica rapa* L (*10*) were washed, soaked 2 hr in distilled water at 25°C, and placed in petri dishes on filter paper moistened with 6 ml of distilled water. The dishes were then slanted in racks providing a 45° angle and incubated in the dark at 25°C for 22 to 24 hr. Twenty seedlings with straight roots of equal length (as measured by calipers under magnification) were arranged in one or two rows in the upper two-thirds of petri dishes on filter paper moistened with 5 ml of 6.7 mM phosphate buffer (*p*H 5.9) that contained the various compounds to be tested. Identical control seedlings in dishes with buffer alone were included in each experiment. The tests were incubated as before for 24 hr, and root growth was then measured by placing the seedlings on millimeter graph paper.

The results of a typical experiment with D-54 · HCl and 4-aminopteroylglutamic acid (4-APGA), an analog of pteroylglutamic acid (PGA) known to inhibit plant growth (*11*), are illustrated in Fig 1. The addition of 100 μg/ml of D-54 · HCl to the buffer resulted in marked inhibition of root growth, and lesser concentrations were progressively less effective. The inhibitory activity of D-54 · HCl was considerably less than that of 4-APGA, as is indicated by the inhibition obtained with 2 μg/ml of 4-APGA. The data derived from several such experiments are summarized in Fig. 2, where the mean growth increment of treated seedlings is plotted as a percentage of the mean growth increment of control seedlings, which is designated as 100 percent. The inhibition of root growth by D-54 · HCl, as with 4-APGA, exhibited a linear increase in response to logarithmic increases in concentration of inhibitor.

The 1,2-dihydro-*s*-triazines, like 4-APGA, interfere with the conversion of PGA to citrovorum factor (CF) in bacterial (*3*) and mammalian liver systems (*12*). However, the mechanisms of action of the two classes of inhibitors differ, the 1,2-dihydro-*s*-triazines inhibiting a coenzyme I-dependent system concerned with the biological reduction of PGA (*13*). Thus, in appropriate microbiological systems, reduced PGA (*14*), coenzyme I or its precursors, CF, thymine or thymidine, but not unaltered PGA, reversed D-54 · HCl inhibition (*3, 13*). Accordingly, analogous reversal experiments were conducted with these various metabolites in the seedling system. These experiments were identical with those previously described, except that the metabolites were added to the buffer simultaneously with a constant concentration of inhibitor (10 μg/ml of D-54 · HCl) that resulted in about 50-percent inhibition of the mean growth increment. The extent of reversal was computed by determining the mean growth increments resulting from the addition of a given metabolite to such inhibited systems.

Inhibition by D-54 · HCl (Table 1), like that of 4-APGA in plant systems, was not reversed by PGA (*11*) or a precursor (PABA) but was reversed inconsistently by reduced PGA, partially by nicotinic acid or coenzyme I, and more effectively by CF, thymine or thymidine, as has been observed in bacterial systems. This pattern of reversal is consistent with previous observations, suggesting that, as is the case with certain bacteria, the conversion of PGA to CF in this seedling system involves a coenzyme I—

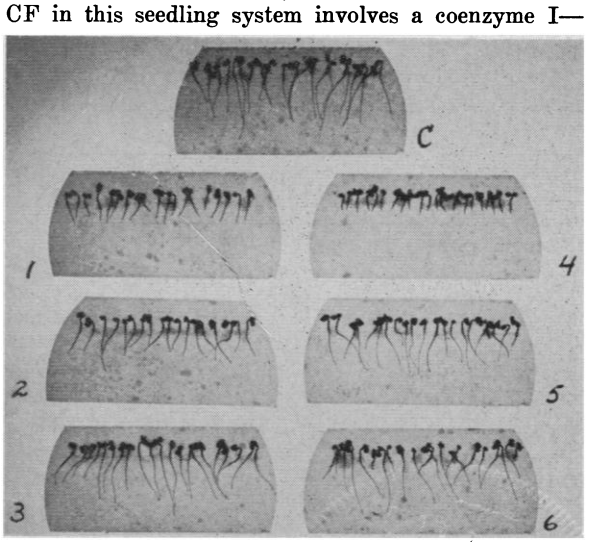

Fig. 1. Inhibition of root growth of seedlings of *Brassica rapa* L by D-54 · HCl and 4-APGA; (1, 2, 3) 2.0, 0.2, and 0.02 μg/ml of 4-APGA, respectively; (4, 5, 6) 100, 10, and 1 μg/ml of D-54 · HCl, respectively; *C*—untreated control.

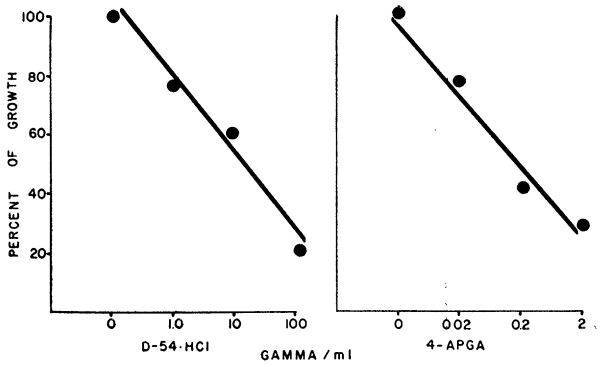

Fig. 2. Dose-response relationships.

dependent reduction that can be blocked effectively by D-54 · HCl. The reversal of this inhibition by thymine or thymidine indicates that as in bacterial systems, the metabolism of PGA is concerned with the biosynthesis of precursors of nucleic acid.

In other experiments, cytological studies on root meristems of *Vicia faba* seedlings and *Trillium erectum* rhizomes exposed to 0.1 to 0.2 μg/ml of 4-APGA or 1 to 2 μg/ml of D-54 · HCl for 24 hr at 25°C in the dark revealed a marked decrease in the number of cells in normal meta,- ana-, and telophase, and an accumulation of overaged prophase cells. Study of similar roots allowed to recover in distilled water for several hours following such exposure to D-54 · HCl indicated that a significant number of cells had been damaged permanently, as is evidenced by the presence of dying pyknotic cells and aberrant mitoses characterized by "sticky" anaphase bridges. These observations suggest that, like the inhibitory activity of 4-APGA in plant (*11*) and mammalian systems (*15*), the inhibitory activity of D-54 · HCl may be attributed at least in part to interference with normal mitosis.

Table 1. Reversal of D-54 · HCl (10 μg/ml) inhibition of *Brassica rapa* L seedling root growth.

Metabolite*	Amount (μg/ml)	Reversal (%)
p-Aminobenzoic acid (PABA)	1.0	0
PGA	10–1.0	0
Dihydro-PGA†	10–1.0	Inconsistent
CF	0.02	50
Thymine	8–2.0	15–20
Thymidine	0.8	50
	.2	25
Uracil	2.0	0
Nicotinic acid	1.0	10–15
Coenzyme I	50.0	10–15
Thiamine, pyridoxine, and so forth	0.1	0

* Higher concentrations in most instances were toxic, as is evidenced by inhibition of growth in comparable experiments without inhibitor.

† Prepared in these laboratories by E. J. Modest, after O'Dell *et al.* (*14*). Presumably the 7,8-dihydro-derivative.

These data suggest that (i) CF or closely related structures may be the form in which PGA is active in plant tissue, and (ii) D-54 · HCl interferes with nucleic acid metabolism by inhibiting the conversion of PGA to CF in a manner similar to that described in bacterial and mammalian systems.

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Detection of Coronary Atherosclerosis in the Living Animal by the Ergonovine Stress Test

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In studies on cardiac pain (*1*), we became interested in the effects of ergonovine on the coronary circulation in patients with effort angina and a normal resting electrocardiogram. Under these conditions, the ergonovine stress test (*2*) may produce alterations in the electrocardiogram indicative of coronary artery disease. The question arose whether the diagnostic value of this test as noted in man likewise applies to the living animal for detection of experimental coronary atherosclerosis. At present, pathologic changes in the coronary arteries can be ascertained only after sacrificing the animal. Electrocardiograms in the atherosclerotic dog (*3*) and chick (*4*) taken in the absence of additional stress on the coronary circulation have not shown significant abnormalities.

For this study (*5*), adult male rabbits fed a diet that contained 2 percent cholesterol, together with control animals fed a stock diet and maintained under otherwise identical conditions, were used (*6*). Series A comprised 16 rabbits kept on the diets for 11 wk, and series B, 18 rabbits kept for 20 to 22 wk on the diets, as shown in Table 1. The ergonovine stress test was carried out once on rabbits of series A, dur-